New insight into cataract formation – enhanced stability through mutual attraction

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Small-angle neutron scattering experiments and molecular dynamics simulations combined with an application of concepts from soft matter physics to complex protein mixtures provide new insight into the stability of eye lens protein mixtures. Exploring this colloid-protein analogy we demonstrate that weak attractions between unlike proteins help to maintain lens transparency in an extremely sensitive and non-monotonic manner. These results not only represent an important step towards a better understanding of protein condensation diseases such as cataract formation, but provide general guidelines for tuning the stability of colloid mixtures, a topic relevant for soft matter physics and industrial applications.

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It has been recognized for some time that apparently unrelated diseases including cataract, sickle-cell and Alzheimer's represent a broad class of 'protein condensation diseases' [1]. While the study of such diseases has traditionally focused on detailed properties of the molecules involved, considerable progress has also been made using statistical and colloid physics. This approach is based on recognizing that a common feature of protein condensation diseases is attractive interaction between specific biological molecules leading to dense phases [1], which compromise cell and organ function. A subtle interplay between protein attractions, repulsions and entropy can lead to these condensed phases, and the difference between health and disease can hinge on intermolecular interaction changes as small as thermal energy, k_BT. The similar sensitivity of colloidal phase transitions suggests that colloid science tools can help understand the molecular origins of protein condensation diseases, and contribute to developing effective preventative measures. In a complementary fashion, globular proteins have also drawn great attention due to their suitability as model colloids [2, 3, 4]. Proteins have proven useful for investigating the combined effects of short-ranged attractions and hard and/or soft repulsions, on phase transitions, aggregation and cluster formation in a wide range of colloidal suspensions [3, 4].

Cataract, clouding of the eye lens due to light scattering and a leading cause of blindness worldwide, is an important protein condensation disease [1, 5, 6, 7, 8]. Statistical physics and colloid science have helped rationalize eye lens protein solution transparency, liquid structure and thermodynamic properties [7, 8, 9, 10, 11]. Eye lens cells contain concentrated solutions of proteins called crystallins. The three major classes of mammalian crystallins are called α, β, and γ [9, 10]. The lens is normally highly transparent and refractive, but loss of transparency due to protein aggregation or phase separation can lead to cataract. Quantifying and understanding crystallin interactions and their impact on lens transparency is therefore a first step towards possible cataract prevention [6].

The α-crystallins are globular, polydisperse, multi-subunit, 800 kDa proteins with a diameter of about 18 nm, whose interactions are well-described with a simple hard-sphere colloid model [12, 13]. The γ-crystallins are monomeric, with a molecular weight of 21 kDa and a diameter between 3 and 4 nm for γB-crystallin. The discovery of a metastable liquid-liquid phase separation provided evidence for short-range attractions between γ-crystallins, and use of the corresponding colloid model has led to a quantitative description of the phase behavior [11, 14, 15, 16, 17, 18].

Although there are known synergetic effects in crystallin mixtures associated with the chaperone activity of α-crystallin, which can suppress induced aggregation of β- and γ-crystallin [19, 20], little is known about the consequences of interactions between unlike crystallins at high concentrations. Recently, α-crystallin has been found to enhance phase separation of γB-crystallin and lead to partial segregation of proteins by type in the separated phases [21]. Therefore we have started a systematic study of α- and γB-crystallin mixtures up to concentrations found within the eye lens, that combines small-angle neutron scattering (SANS) and molecular dynamics (MD) simulations of a coarse-grained colloidal model, appropriate given the hard cores of these folded, globular proteins. This approach allows for complementary experimental and computational investigation at the required large length scales and high number of proteins (∼30,000 - 60,000).

We prepared different mixing ratios from stock solutions of 230 mg/mL α-crystallin (denoted as C_α=100) and 260 mg/mL γB-crystallin (denoted as C_α=0) in 0.1 M sodium phosphate buffer in D_2O at a pH of 7.1, with 20mM

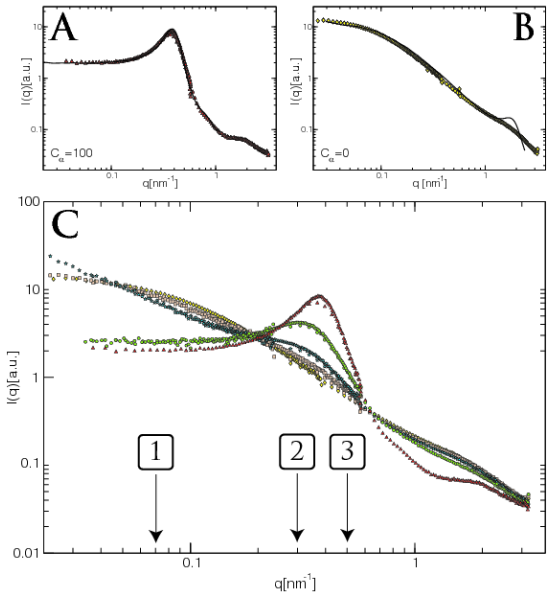

FIG. 1: (Color online) Scattered intensities $I(q)$ of a 230 mg/ml (C_α=100) and a 260 mg/mL (C_α=0) solution and their mixtures. **A**, Experimental $I(q)$ of C_α=100 (open symbols) together with MD computer simulations (full curve). **B**, Experimental $I(q)$ of C_α=0 (open symbols) together with MD computer simulations (full curve). **C**, $I(q)$ of mixtures containing different amounts of the crystallin solutions shown in A and B. Shown are C_α=100 (triangles), C_α=50 (diamonds), C_α=25 (stars), C_α=12.5 (squares), and C_α=0 (circles). Numbers indicate q-values for composition dependence in Fig. 4.

dithiothreitol to inhibit protein oxidation and oligomerization [21]. With these precautions γB-crystallin remained monomeric under all conditions used. We performed SANS using 1 and 2 mm Hellma quartz cells and varied wavelengths, sample-to-detector distances and collimation lengths to cover a q-range of 0.02–3 nm^{-1}. All experiments were performed at a temperature of 25°C, i.e. 10°C above the critical temperature T_c of γB-crystallin in the present buffer. The choice of an experimental temperature close to T_c of γB-crystallin amplifies thermodynamic stability variations due to the presence of alpha crystallin, and thereby enhances sensitivity for discerning effective protein interaction potentials.

We established a model for the protein interaction potentials by comparing MD computer simulations with the experimental scattering intensity $I(q)$ as a function of the scattering vector q. Event driven MD simulations [22] were performed in cubic boxes with periodic boundary conditions for N=32,000 particles for the pure α-crystallin solution and the mixtures, and N=64,000 particles for the pure γ-crystallin solution. The scattering intensities were calculated from the partial structure factors $S_{ij}(q)$ (i,j=α,γ) obtained from independent MD runs and the experimental form factors. In order to account for the experimental smearing, we derived a general resolution function and the scattered intensities were convoluted before comparison with SANS data [23]. From the three different mixing ratios (C_α=50, C_α=25, and C_α=12, where C_α is defined as 100 x [volume of the C_α=100 solution]/[total volume of the mixture]) investigated, C_α=50 closely resembles the natural α- and γ-crystallin concentrations found in the lens nucleus [9]. Therefore the model development is demonstrated on this sample, and its validity is tested by comparison with the remaining samples.

First we determined parameters that provide a quantitative description of $I(q)$ for the individual components. For α-crystallin we used a purely repulsive hard-sphere model and a diameter of d_α = 17.6 nm [13], which results in perfect agreement between experimental and simulated $I(q)$ (Fig. 1A). For C_α=0 the strongly enhanced intensity at low q indicates that a hard-sphere model is not sufficient to describe the interactions between γB-crystallins (diameter d_γ = 3.6 nm) (Fig. 1B). This is a direct consequence of interprotein attraction and proximity to the critical concentration C_c [14, 15, 17], which leads to long-wavelength concentration fluctuations that are a potential source of lens turbidity in cataract [5]. To account for this we added a square-well attractive potential with a range of 0.25 d_γ [16] and a depth $u_{\gamma\gamma} = 1k_BT$, where the temperature T is set to T = 0.7875 (for k_B = 1) for all simulated mixtures. This temperature T has been fixed to reproduce the γ-pure case and results in good agreement between simulated and experimental $I(q)$ in the low-q regime ($q \lesssim 0.047\ nm^{-1}$) relevant for lens transparency (Fig.1B). It is clear that square well potentials are rather unphysical in their shape. However, such potentials have been widely used in studies of colloids with short range potentials due to the fact that the physics of the system is scarcely dependent on the shape of the potential when the range is shorter than the diameter of the particle [24]. Figure 1 indeed demonstrates that simple colloid models are capable of reproducing $I(q)$ for individual α- and γB-crystallin solutions. In a next step we investigated the mixtures. Figure 1C demonstrates the effect of adding alpha crystallin, from C_α=0 to C_α=100. The forward scattering first increases and reaches a maximum for C_α=25. For C_α=50 it becomes highly suppressed, similar to the situation for pure α-crystallin. In a first attempt to simulate the mixtures we assumed the interactions between α- and γB-crystallin to be hard-sphere like. The striking disagreement between the forward scattering of the corresponding simulation with experimental data for C_α=50 is shown in Fig. 2. The enormous increase of the simulated intensity at low q arises most likely from additional depletion-induced attractions known to exist in mixtures of hard spheres with different sizes [25]. Such an effect would increase T_c for liquid-liquid phase separation and result in segregation of the proteins into large domains of α-crystallin-rich and γB-crystallin-rich regions (Fig. 2).

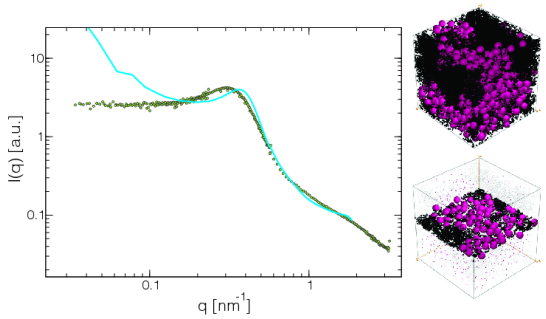

FIG. 2: (Color online) Scattered intensity $I(q)$ of a mixture of α- and γB-crystallin ($C_\alpha = 50$) and MD computer simulation assuming mutual repulsion between unlike proteins. Left, $I(q)$ of C_α=50 (open symbols) together with MD simulation of the system. Right, Snapshot from MD simulation shown on the left. Assuming repulsive hard sphere interactions only between α- (grey spheres) and γB-crystallins (black dots) leads to a strong segregation by protein type and the corresponding large density fluctuations would lead to a loss of transparency for visible light. Also shown is a slab of the box for this snapshot.

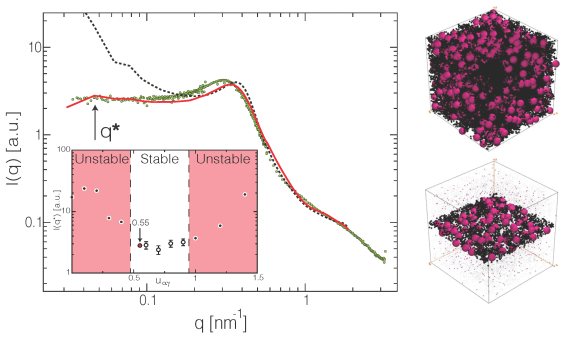

FIG. 3: (Color online) $I(q)$ of an α- and γB-crystallin mixture ($C_\alpha = 50$) and simulations incorporating mutual attraction. Left, SANS data from Fig. 2 (open symbols) and simulations without (dashed curve) and with added short-range attractions (full curve; $u_{\alpha\gamma} = 0.55 k_B T$) between unlike proteins. Inset: $I(q^* = 0.0467$ nm$^{-1})$ from simulations as a function of $u_{\alpha\gamma}$. The mixtures become unstable for $u_{\alpha\gamma} \lesssim 0.5 k_B T$ and $\gtrsim 1 k_B T$. Right, Snapshot and slab representation of the box for this situation as in Fig. 2. The solution is homogeneous and does not give rise to increased light scattering.

As a consequence, light scattering would have increased and transparency would have been lost, in contrast to the experimental data and the visual appearance of the sample. Such effects are in fact manifest in α-γB mixtures at temperatures lower than those investigated here [21]. However, the low forward scattering intensity $I(0)$ of the present SANS data indicates that they are suppressed under the present conditions, closer to body temperature. Nature must have found ways to circumvent these long-wavelength fluctuations and the accompanying clouding of the eye lens that would have been caused by hard sphere interactions between α- and γB-crystallins, and we can speculate that an additional attraction could exist between these unlike proteins.

In a second attempt we thus added an attractive interaction between α- and γB-crystallins, assuming that the attractive part of the potential has the same range as that used for the attraction between γB-crystallins. The depth of this interspecies attractive potential, $u_{\alpha\gamma}$, was chosen to reproduce $I(q)$ and is roughly one half of the γB-γB-attraction. The simulated $I(q)$ (Fig. 3) indeed perfectly reproduces the SANS data throughout the entire q-range. Use of the novel attractive term leads to good agreement between simulations and SANS for all mixing ratios (Fig. 4). A snapshot of the C_α=50 simulation (Fig. 3) suggests a qualitative explanation: attractions between unlike proteins counterbalance and efficiently suppress segregation of the two proteins into large domains [26]. Thus transparency of concentrated crystallin mixtures is maintained by introducing weak, short-range attractions between α- and γ-crystallins, which considerably decrease T_c and the corresponding critical fluctuations at a given temperature.

We also performed simulations in which the attraction between α and γB was further increased, to above 100% of the γB-γB-attraction. Such stronger attractions again resulted in enhanced instability (inset of Fig. 3). Thus the stability of these high concentration crystallin mixtures depends on α-γB-attraction in a manner that is both extremely sensitive and non-monotonic. Such non-monotonic effects are a common feature of ternary liquid mixture phase separation [21, 27].

There are studies suggesting that γ-crystallins inhibit the age-related aggregation of α-crystallins [28]. On the other hand, numerous investigations have suggested that inhibition of age- or heat-induced aggregation of γ- and β-crystallins is associated with the chaperone activity of α-crystallins [19, 20, 29]. In contrast to these stabilizing effects of α-γ interactions, it has recently been suggested that the observed increase in the non-covalent association of γ-crystallins to α-crystallins in aging bovine lenses might adversely affect the optical properties of the aged and/or cataractogenic lens [30], since strong attractions could lead to larger aggregates and increased scattering of light. While such a mechanism of increased protein-protein association is a possible route towards cataract [30], our experiments and simulations provide evidence that a weak and short-range attraction between α- and γB-crystallins can potentially enhance

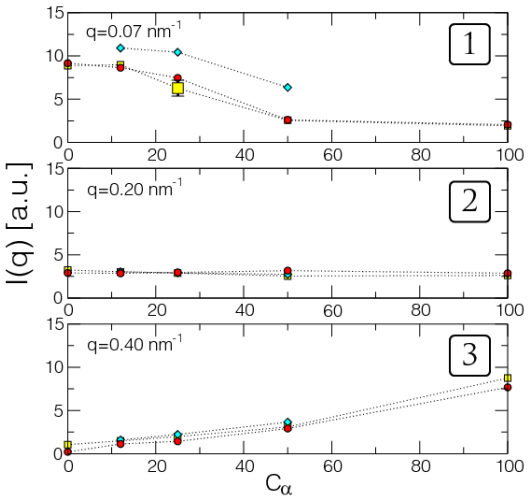

FIG. 4: (Color online) Comparison of $I(q)$ obtained from simulations and SANS as a function of α-crystallin mixing ratio (C_α) in the α-γB mixtures. The predicted low-q portion of $I(q)$ is very sensitive to alpha-gamma mutual attraction, unlike the higher-q portions. $I(q)$ at different C_α are from SANS (circles) and simulations without (diamonds) and with (squares) mutual attractions. Shown are data at three scattering vectors, q = 0.07, 0.2, and 0.4 nm^{-1} (the numbered labels refer to the arrows in Fig. 1C). Unless explicitly plotted, error bars are equal or smaller than the symbols.

transparency in healthy lenses.

It is intriguing that a variety of specific molecular mechanisms could potentially underly this 'stabilization due to mutual attraction', since a simple colloidal α-γB attraction is sufficient to rationalize our observations. Due in part to its small magnitude, the molecular origin of the inferred, net short-range attraction between α- and γB-crystallins remains a challenging and open question, as it is for short-range attractions of many globular proteins including γ-crystallin and lysozyme [2, 31].

It has long been recognized that uniform packing of crystallins at high concentrations, termed short-range order, suppresses index of refraction fluctuations and reduces light scattering [7]. The chemically specific origins of short-range order are key to understanding lens transparency. Not only do crystallins vary widely in short-range order properties, sensitivity to inter-crystallin interactions makes short-range order not a simple combination of individual crystallin properties, as shown here for α- and γB-crystallins. Thus to understand transparency of crystallin mixtures, high concentration short-range order study, using tools of liquid-state and colloid physics, remains a needed complement to low concentration investigations.

This investigation has provided new insight into the stability and optical properties of lens protein mixtures that may help understand cataract formation. The present results also suggest mechanisms to tune the stability of classical colloidal mixtures, a topic of considerable industrial importance. This demonstrates the importance of a colloid-oriented approach to proteins as a means of obtaining insight into questions of biological, medical, as well as fundamental soft matter physics relevance.

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[1] G. B. Benedek, Invest Ophthalmol Vis Sci **38**, 1911 (1997).
[2] R. Piazza, Curr. Opin. Colloid Int. Sci. **5**, 38 (2000).
[3] A. Stradner et al., Nature **432**, 492 (2004).
[4] F. Cardinaux et al., Phys. Rev. Lett. (2007, accepted for publication).
[5] G. B. Benedek, Applied Optics **10**, 459 (1971).
[6] G. B. Benedek et al., Prog. Retin. Eye. Res. **18**, 391 (1999).
[7] M. Delaye and A. Tardieu, Nature **302**, 415 (1983).
[8] H. Bloemendal et al., Prog. Biophys. Mol. Biol. **86**, 407 (2004).
[9] R. J. Siezen et al., Proc Natl Acad Sci U S A **82**, 1701 (1985).
[10] F. Vérétout, M. Delaye, and A. Tardieu, J. Mol. Biol. **205**, 713 (1989).
[11] Schurtenberger et al., Phys. Rev. Lett. **63**, 2064 (1989).
[12] A. Tardieu, Int J Biol Macromol **22**, 211 (1998).
[13] S. Finet and A. Tardieu, J. of Cryst. Growth **232**, 40 (2001).
[14] J. A. Thomson et al., Proc Natl Acad Sci U S A **84**, 7079 (1987).
[15] M. L. Broide et al., Proc Natl Acad Sci U S A **88**, 5660 (1991).
[16] A. Lomakin, N. Asherie, and G. B. Benedek, J. Chem. Phys. **104**, 1646 (1996).
[17] M. Malfois et al., J. Chem. Phys. **105**, 3290 (1996).
[18] A. Stradner, G. M. Thurston, and P. Schurtenberger, J. Phys.: Condens. Matter **17**, S2805 (2005).
[19] J. Horwitz, Proc Natl Acad Sci U S A **89**, 10449 (1992).
[20] T. Putilina et al., J. Biol. Chem. **278**, 13747 (2003).
[21] G. M. Thurston, J. Chem. Phys. **124**, 134909 (2006).
[22] D. C. Rapaport, *The Art of Molecular Dynamic Simulation* (Cambridge University Press, 1995).
[23] N. Dorsaz et al., to be submitted.
[24] G. Foffi et al., Phys. Rev. E **65**, 031407 (2002).
[25] Mendez-Alcaraz and R. Klein, Phys. Rev. E **61**, 4095 (2000).

[26] A. A. Louis et al., Phys. Rev. E **65**, 061407 (2002).
[27] J. Meijering, Philips Research Reports **5**, 333 (1951) and **6**, 183 (1951).
[28] H. Mach et al., J. Biol. Chem. **265**, 4844 (1990).
[29] V. Pigaga and R. A. Quinlan, Exp. Cell Res. **312**, 51 (2006).
[30] J. Peterson, G. Radke, and L. Takemoto, Exp. Eye. Res. **81**, 680 (2005).
[31] R. Piazza, Curr. Opin. Colloid Int. Sci **8**, 515 (2004).
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